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Combined effect of stringent or relaxed response, temperature and *rom* function on the replication of pUC plasmids in *Escherichia coli**

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pUC Plasmids, commonly used vectors in molecular cloning and ColE1-type replicons, are models for investigation of DNA replication. Regulation of initiation of the ColE1-type plasmid replication in *Escherichia coli* cells is based on the function of two plasmid transcripts: RNA I and RNA II (for review see ref. [1]). RNA II is a pre-primer RNA which forms a persistent hybrid with its DNA template. RNase H recognizes and cleaves this hybrid, generating the mature primer. The primer is the target for DNA polymerase I which adds deoxynucleotides to its 3'-OH end. RNA I is a negative regulator of the replication initiation, as by hybridizing to the pre-primer RNA it inhibits hybrid formation between RNA II and its DNA template. Some ColE1-type plasmids (but not pUC plasmids) contain the *rom* gene which encodes a second negative regulator of replication initiation: the Rom protein which enhances RNA I–RNA II binding.

pUC Plasmids [2] are pBR322 derivatives that replicate at a copy number several fold higher than that of the parent plasmid. Recently, Lin-Chao *et al.* [3] found that the high copy number of the pUC plasmids results from a Rom-suppressible point mutation in RNAII. This phenomenon is also dependent on temperature. On the other hand, the effect of stringent response (the bacterial response to amino acid starvation) on pBR322 plasmid DNA replication has been demonstrated [4, 5]. pBR322 Replication in amino acid-starved wild type bacteria was inhibited whereas in *relA*$^-$ mutants in the same conditions plasmid DNA was amplified.

We confirmed the previous observation [3] that the content of pUC plasmid in *E. coli* cells was dependent on temperature. Moreover, we found that in *relA*$^-$ mutant the relative plasmid content per bacterial mass was several fold higher than in *relA*$^+$ strain (Table 1). The *relA*

Table 1

Relative pUC19 plasmid content per bacterial mass in E. coli relA$^+$ *and relA*$^-$ *strains exponentially growing in minimal medium 2 [5] at 30°C and 43°C.*

Wild type (*relA*$^+$) strain CF1648 [6] and *relA*$^-$ mutant CF1652 [6] were used. The relative amount of plasmid DNA was estimated as described by Węgrzyn *et al.* [7] except that lysis by alkali [8] was applied and the amount of DNA in the bands on the electrophoregram was estimated using the UVP E.A.S.Y. densitometry system. The value obtained for *relA*$^+$ strain at 30°C was taken as 1.

Strain	Relative plasmid content at:	
	30°C	43°C
relA$^+$	1	12.3
relA$^-$	10.4	124.8

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gene codes for the enzyme (ATP:GTP 3'-pyrophosphotransferase) producing pppGpp (guanosine 5'-triphosphate-3'-diphosphate), the precursor of ppGpp (guanosine 5'-diphosphate-3'-diphosphate) which is the effector of the stringent response. ppGpp Interacts with RNA polymerase [9] and this interaction leads to changes in efficiency of transcription from many promoters. In wild type cells, even in normal growth conditions, ppGpp is kept on the basal level whereas *rel*A$^-$ mutants do not accumulate this nucleotide. Therefore, the results presented in Table 1 indicate that ppGpp somehow regulates pUC19 plasmid DNA replication. Lin-Chao & Bremer [10] concluded that the promoters for RNA I and RNA II syntheses are not under stringent control. On the other hand, it was proposed [11] that regulation of the level of active RNA I molecules might occur by interactions of RNA I with tRNAs which possess complementary sequences to RNA I. Since tRNA synthesis is inhibited during stringent response [12], ppGpp might decrease indirectly the efficiency of initiation of ColE1-type plasmid DNA replication. If this is true, the effect of ppGpp on the replication of the pUC plasmid should be more distinct during amino acid starvation. We investigated the replication of pUC19 plasmid in isoleucine-starved *rel*A$^+$ and *rel*A$^-$ strains at different temperatures. In addition, we introduced the *rom* gene into pUC19 plasmid. Amino acid starvation provokes inhibition of bacterial growth. Thus, if the replication of plasmid DNA were stopped, the relative amount of plasmid DNA per cell mass should stay constant. The increase of the plasmid content (amplification of plasmid DNA) indicates prolongated plasmid DNA replication. In the case of pUC19, the amplification of plasmid DNA was observed in both isoleucine starved *rel*A$^+$ and *rel*A$^-$ bacteria at 37°C, only in *rel*A$^-$ strain at 30°C and only in *rel*A$^+$ strain at 43°C (Table 2). In the presence of the *rom* gene the amplification was observed only in isoleucine-starved *rel*A$^-$ mutant at higher (37°C and 43°C) temperatures (Table 2).

We assume that the hybridization between RNA I and RNA II is more efficient at a lower temperature (30°C) in the case of pUC19 replicon, as it was proposed recently [3]. Therefore, the negative effect of ppGpp on pUC19 replication, combined with the above mentioned influence of temperature, appears to provoke inhibition of DNA replication of this plasmid in *rel*A$^+$ strain at 30°C. The high plasmid content in *rel*A$^-$ strain at 43°C (Table 1) may be the reason why we did not observe the amplification of pUC19 plasmid in these conditions. We consider that introduction of the *rom* gene into pUC19 plasmid results in increased efficiency of RNA I–RNA II hybridization which is mediated by the Rom protein. Thus, the replication of the pUC plasmid containing the *rom* gene (plasmid pWUZ2) is inhibited during amino acid starvation at all studied temperatures (30°C, 37°C and 43°C) in *rel*A$^+$ strain and at 30°C in *rel*A$^-$ mutant. The *rom* function is not sufficient for inhibition of pWUZ2 plasmid DNA replication in isoleucine-starved *rel*A$^-$ bacteria at higher temperatures.

In summary, we conclude that: (i) ppGpp indirectly inhibits the replication of the pUC plasmid DNA; (ii) the replication of the pUC plasmid in amino acid-starved cells is tempera-

Table 2

Replication of pUC19 and pWUZ2 plasmids in isoleucine-starved relA$^+$ and relA$^-$ E. coli strains at different temperatures.

pUC19 Plasmid was described earlier [2]. pWUZ2 Plasmid was constructed by ligation of *Nde*I–*Eco*RI fragment (containing *bla* gene and the origin of replication) from pUC19 with *Nde*I–*Eco*RI fragment (containing *tet* and *rom* genes) from pBR322. Amplification factor was calculated as the ratio of plasmid content per bacterial mass three hours after the induction of isoleucine starvation, to the plasmid content per bacterial mass in the nonstarved culture. The relative amount of plasmid DNA was estimated as described in Table 1. Isoleucine starvation was provoked by addition of L-valine up to 1 mg/ml according to Węgrzyn *et al.* [7]. Bacterial strains (*rel*A$^+$ and *rel*A$^-$) are described in Table 1.

Plasmid	Amplification factor					
	30°C		37°C		43°C	
	*rel*A$^+$	*rel*A$^-$	*rel*A$^+$	*rel*A$^-$	*rel*A$^+$	*rel*A$^-$
pUC19	1.5	3.3	2.4	2.3	2.8	1.0
pWUZ2	0.9	1.2	0.9	2.5	0.8	2.2

ture dependent, as the rate of RNA I–RNA II hybridization is lower at higher temperatures; (iii) *rom* gene plays an important regulatory role in the stringent control of replication of the pUC plasmid DNA.

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